

Research Note

Phenolic Compounds in Cork-Closed Bottle-Fermented Sparkling Wines

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Bottle fermented sparkling wine in South Africa is known as *Méthode Cap Classique* which is based on the method used in France for Champagne. The use of cork, instead of a crown cap during the second fermentation in sparkling wine was investigated for its effect on the phenolic profile of wines. Phenolic acids susceptible to migration from cork into wine were studied in two-disc corks from three different commercial suppliers, coded as Cork A, Cork R and Cork C and a crown cap closure. Gallic, caftaric, caffeic and *p*-coumaric acids were quantified in all samples using a liquid chromatographic technique. Physicochemical parameters were also measured in the wine using a spectrophotometric technique. Total acidity and pH were not significantly different among the wines. Cork R wines were however significantly different in alcohol. Residual sugar for all samples was below the limit of detection. Gallic acid was significantly highest in Cork A wines, which indicates the contribution of Cork A to the concentration of this compound in the wine. Different cork types are assumed to release different concentrations of phenolic compounds. This may be due to differences in surface roughness of cork that would increase the surface area in contact with the wine. Therefore, corks from different origins (suppliers) could be used to bring about subtle differences to the wine.

INTRODUCTION

Méthode Cap Classique (MCC) is the South African name for bottle fermented sparkling wine. Although it is the same method used in France for Champagne production, the *Organisation internationale de la vigne et du vin* (OIV) regulations stipulate that sparkling wine produced outside of the Champagne region of France may not be called Champagne (Amerine *et al.*, 1980).

Bottle fermented sparkling wine has two stages of production, i.e. primary (first) fermentation and second fermentation. In the first fermentation, wine is produced in the same manner as still wines. After blending, a mixture of the base wine, sugar and yeast is bottled with a crown cap. Crown cap closures have the ability to contain pressure and is used in bottle fermented sparkling wine production due to ease of use and automation. Prior to the use of modern crown caps in the 1960s, a cork secured with a metal staple (*Agrafê*) was used as a closure (Anonymous, 2020). The second fermentation occurs in the bottle and after a mandatory maturation period, the wine is clarified (riddling) and disgorged, during which the crown cap is removed.

In the final stages of sparkling wine production, the wine (still in the same bottle it was fermented in) is closed with a cork and secured with a wire hood (Amerine *et al.*, 1980). The body of the sparkling wine cork generally consists of agglomerated cork with one or two natural cork discs, which are in contact with the wine (Rives *et al.*, 2012). Cork is a suitable closure for sparkling wine due to its impermeability to fluids and to a lesser extent air (preventing wine oxidation), compressibility, flexibility and elasticity (Silva *et al.*, 2005; Prat *et al.*, 2011).

A study by Marin *et al.* (2007) showed that still wines bottled under cork were of higher quality than wines bottled with screw cap closures. Cork has been associated with low molecular weight compounds such as phenolic acids, as well as the presence of more complex structures such as tannins and volatile compounds (Fernandes *et al.*, 2009). Therefore, certain cork compounds (non-volatile and volatile) can be extracted from cork and migrate into wine during bottle maturation when the wine is in contact with the cork disc, and subsequently alter the organoleptic properties and

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chemical composition of the wine (Fernandes *et al.*, 2009; Lopes *et al.*, 2009; Hopfer *et al.*, 2012; Pinto *et al.*, 2019).

Phenolic acids that permeate from cork into wine, result in increased concentrations of phenolic acids in the wine, which can affect mouthfeel, i.e. gallic acid and ellagic acid (Gabielli *et al.*, 2016). However, the initial chemical compounds derived from grapes and formed during fermentation are the principal contributors to wine mouthfeel, flavour and overall quality (Morena *et al.*, 2016).

Studies by Mitić *et al.* (2010) and Azevedo *et al.* (2014) reported that phenolics can improve the quality of wine, i.e. mouthfeel, colour and antioxidant content, but vinification techniques still play an important role in the phenolic composition of wine and can be modulated by wine makers to create desired characteristics in the wine (Lingua *et al.*, 2016). One of these techniques would be the use of cork instead of more inert closures such as crown caps.

In the Champagne wine region of France, some producers have continued the tradition of using a cork closure during the second fermentation, and never switched to using crown caps for their premium products (D. Bunner, House of Bollinger, Ay, Champagne, personal communication, 2018) due to a perceived positive effect on the sensory properties of the wine, despite the risk of cork-taint (2,4,6-trichloroanisole). This has led to a renewed oenological interest in using cork as a primary closure for MCC, although there is only anecdotal evidence that it is advantageous to the final product.

The aim of the study was to investigate the effect of cork closures from three different cork suppliers in South Africa on the phenolic compounds in MCC Brut sparkling wines during the second fermentation and maturation. The effect of cork on the physicochemical characteristics was also investigated.

MATERIALS AND METHODS

Wine samples and treatments

Méthode Cap Classique Brut sparkling wines from the 2014 vintage undergoing in-bottle-fermentation were sourced from a commercial wine cellar in Robertson (33.8021°S, 19.8875°E), South Africa, after the wine had been in contact with the yeast lees for 48 months at 15°C. Three different two-disc corks, i.e. Cork A (*Amorim* Cork South Africa), Cork R (RR Cork Suppliers, South Africa), Cork C (Cape Cork Supply, South Africa) and a crown cap (African Cellar Suppliers) closures were used (Table 1). The wines had been bottled from the same tank, and sealed with the agglomerated two-disc cork closures and a crown cap as control.

Sample preparation and physicochemical parameters

The wines (analysis of 3 bottles per treatment, i.e. triplicate) for each treatment were chilled overnight at 4°C prior to opening. Once the bottles were opened, the wines were centrifuged (Avanti®, Beckman-Coulter, Darmstadt, Germany) at 6000 rpm at 15°C for 5 min to degas and clarify the wines.

Physicochemical characteristics, i.e. pH, total acidity (TA, tartaric acid equivalents), and alcohol (% v/v) and residual sugar (g/L) (RS) were measured using an OenoFoss™ wine analyser (FOSS, Hellerød, Denmark) with the instrument's internal calibrations.

Liquid chromatographic analysis

The RP-HPLC determination of phenolic acids was performed using an Agilent model 1260 HPLC system (Agilent Technologies, California, USA). The system was equipped with an auto-sampler and a photodiode array detector. A polymer reversed-phase analytical column (PLRP-S 100 Å, 5 µm, 250 x 4.6 mm) with polystyrene divinylbenzene as a stationary phase was used for compound separation (Varian, Polymer Laboratories, Palo Alto, California, USA). A gradient mobile phase programme was used for compound elution. Mobile phase A consisted of water/phosphoric acid (985:15 v/v) with a pH of ca. 1.35, and mobile phase B consisted of water/phosphoric acid/ acetonitrile (185:15:800 v/v/v) with a pH of ca. 1.25. The following gradient mobile phase programme was used for compound separation: 94% of mobile phase A was used initially at 0 min, 94% to 69% of mobile phase A at 73 min; 69% to 38% of mobile phase A at 78 min; 38% to 94% of mobile phase A at 90 min. The column and the system were equilibrated for 20 minutes after each analysis run time of 90 min to revert to the starting conditions. The flow rate was 1 mL/min. Separation of the compounds was carried out at ca. 25°C. Individual phenolic acids in the wines were quantified using peak areas at 316 nm. The identification of phenolic acids in the wines was confirmed by their relative retention times based on available phenolic acid reference standards and UV-visible absorption characteristics (Stefova *et al.*, 2003, De Villiers *et al.*, 2011; Minnaar *et al.*, 2015). Quantification was based on calibration curves of commercial gallic, caffeic, caftaric and *p*-coumaric acid reference standards (Merck [Pty] Ltd, Johannesburg, South Africa). Wine sample aliquots of 2 mL were filtered through 0.45 µm nylon membrane syringe filters prior to analysis. A 50 µL sample filtrate was injected onto the HPLC column. Replicate samples were analysed on the same day.

TABLE 1
Closure type used for sealing the MCC Brut sparkling wines.

Closure/treatment	Supplier	Type
Cork A	¹ <i>Amorim</i> Cork South Africa	Two-disc cork
Cork R	RR Cork Suppliers (SA) (Pty) Ltd	Two-disc cork
Cork C	Cape Cork Supply SA	Two-disc cork
Crown cap control	African Cellar Suppliers	Metal crown cap

¹*Amorim* Cork South Africa is a subsidiary company of the *Amorim* Group in Portugal

Spectrophotometric analysis

Total phenolic acids, flavanols and flavonols were quantified using a spectrophotometric method (Minnaar *et al.*, 2018). An UV-vis Aurius Model CE2021 spectrophotometer (Cecil, Cambridge, UK) was used with the wavelength scan programme mode to determine the maximum wavelength absorbance for phenolic acids (316 nm), total flavonols (360 nm) and total flavanols (279 nm) (Minnaar *et al.*, 2018) using pure *p*-coumaric acid (phenolic acids), and quercetin (flavonols) and gallic acid (flavanols). Calibration curves established from reference standards were used to determine the concentrations in the matrix. Concentrations were expressed mg quercetin equivalents (mg QUE), mg *p*-coumaric acid equivalents (mg PCAE) and mg gallic acid equivalents (mg GAE) per litre for total flavonols, total phenolic acids and total flavanols, respectively. The spectrophotometric technique used is relatively non-specific, but does give an indication of the presence of compounds measured at maximum absorbance.

Statistical analysis

Physicochemical and phenolic data were subjected to analysis of variance (ANOVA) using General Linear Models Procedure of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). Shapiro & Wilk (1965) test verified normality of standardized residuals. Fisher's least significant difference was calculated at a 5% ($p = 0.05$) probability level to compare treatment means. Principal component analysis (PCA) was performed using phenolic acids and total phenolics

as variables. Principal component analysis, employing the correlation matrix, was performed using XLSTAT (Version 2015.1.03.15485) to elucidate the associations amongst treatments and observed variables.

RESULTS AND DISCUSSION

Physicochemical parameters

Significant differences in physicochemical parameters measured in the wines and control samples were not evident except alcohol and pH (Table 2). Alcohol in Cork R wines were significantly lower than Cork C, and Cork A wines as well as the control wines. Alcohol in Cork A, Cork C and control wines did not differ significantly. The decrease of alcohol in cork wines, compared to the crown caps wines cannot at this stage of the investigation be explained.

The mean pH values of Cork R wines were significantly higher compared to the mean values of the control wines and Cork C wines, but not different from Cork A wines, and the mean pH values of Cork A wines did not differ significantly from the control wines.

Residual sugar levels were below the limit of detection. The lack of significant differences in RS and alcohol (except Cork R wines) suggest that fermentation continued in the same way, and was therefore not affected by the closures.

Phenolics

Generally, no significant treatment differences were found for most of the phenolic acids (Table 3). However, Cork A wines were significantly higher in gallic acid, compared to

TABLE 2

Mean values for physicochemical parameters measured in MCC Brut sparkling wines.

Parameters measured	Wine treatments			
	¹ Cork A	¹ Cork C	¹ Cork R	² Crown cap (control)
TA (g/L)	³ 6.46a ± 0.03*	6.50a ± 0.19	6.27a ± 0.28	6.54a ± 0.07
pH	3.37ba ± 0.01	3.35b ± 0.04	3.42a ± 0.03	3.35b ± 0.04
RS (g/L)	⁴ BLD	⁴ BLD	⁴ BLD	⁴ BLD
Alcohol (%)	11.69a ± 0.24	11.92a ± 0.42	10.77b ± 1.28	12.15a ± 0.16

¹Different cork suppliers in South Africa (Table 1); ²African Cellar Suppliers (supplier of crown cap); ³Mean values ± standard deviation of physicochemical parameters in MCC Brut sparkling wines and ANOVA comparative test results. ⁴Below limit of detection. *Means with different letters in the same row are significantly different ($p = 0.05$).

TABLE 3

Mean values for phenolic acids (mg/L) measured in MCC Brut sparkling wines.

Phenolic acids	Wine treatments			
	¹ Cork A	¹ Cork C	¹ Cork R	² Crown cap (control)
Gallic acid	³ 16.18a±0.41*	14.86b0.18	14.90b±1.21	15.23ab±0.22
Caftaric acid	8.03a±0.30	7.66a±0.20	7.97a±0.20	7.69a±0.02
Caffeic acid	5.44a±0.14	5.40a±0.01	5.50a±0.05	5.45a±0.06
<i>p</i> -Coumaric	4.33a±0.33	4.42a±0.22	4.42a±0.11	4.52a±0.09

¹Different cork suppliers in South Africa (Table 1); ²African Cellar Suppliers (supplier of crown cap); ³Mean values ± standard deviation of gallic, caftaric, caffeic and *p*-coumaric acids in MCC Brut sparkling wines and ANOVA comparative test results. *Means with different letters in the same row are significantly different ($p = 0.05$).

Cork C and Cork R wines but were not significantly different from control wines. Though statistically significant, this may not be relevant from a practical point of view unless sensory analyses proves the contrary.

Cork A wines were not significantly different from the control wines. Gallic acid in Cork C and Cork R wines decreased relatively to the control wines. Caftaric, caffeic and *p*-coumaric acids in Cork A, Cork C and Cork R wines were not significantly different than the control wines.

The main source of wine phenolics is grapes; however, Mazzoleni *et al.* (1998) and Verea *et al.* (2001) reported the presence of gallic acid and other low molecular weight phenolics in cork, but limited data related to the phenolic compound extractability and migration into wine are available. In another study, Gabrielli *et al.* (2016) showed that in still wines, phenolic acids permeating from cork into wine, resulted in an increase in concentrations of phenolic

acids. Contrary to work by Gabrielli *et al.* (2016), results of this study showed that generally, a significant increase in individual phenolic acids from cork into the wines was not evident.

Cork wines were not significantly different from the control wines in total phenolic acids (Table 4). Although flavonols were significantly lower in Cork A and Cork C wines in comparison to the control wines, the differences in flavanols of the cork wines, compared to the control wines were not significant. Even without statistical significance for flavanols, and relatively low differences, the results still show a tendency for a slight decrease of the flavonol and flavanol contents in cork sparkling wines, compared to the crown cap wines. Oxidative polymerisation of monomeric phenolic compounds (e.g. flavonols and flavanols) can occur, which would result in a decrease of these compounds (Lopes *et al.*, 2009).

TABLE 4

Mean values for total phenolic acids (mg *p*-coumaric acid equivalents/L), flavonols (mg quercetin equivalents/L) and flavanols (mg gallic acid equivalents/L) measured in MCC Brut sparkling wines.

Phenolic classes	Wine treatments			
	¹ Cork A	¹ Cork C	¹ Cork R	² Crown cap (control)
Phenolic acids	³ 11.85a±0.34*	11.55a±0.59	11.28a±0.58	11.48a±0.54
Flavonols	24.71a±1.07	24.70a±1.79	25.91ab±0.68	26.38b±0.99
Flavanols	21.86a±0.21	21.31a±1.09	21.73a±0.45	22.04a±0.05

¹Different cork suppliers in South Africa (Table 1); ²African Cellar Suppliers (supplier of crown cap); ³Mean values ±standard deviation of phenolic acids, flavonols and flavanols in MCC Brut sparkling wines and ANOVA comparative test results. *Means with different letters in the same row are significantly different ($p = 0.05$).

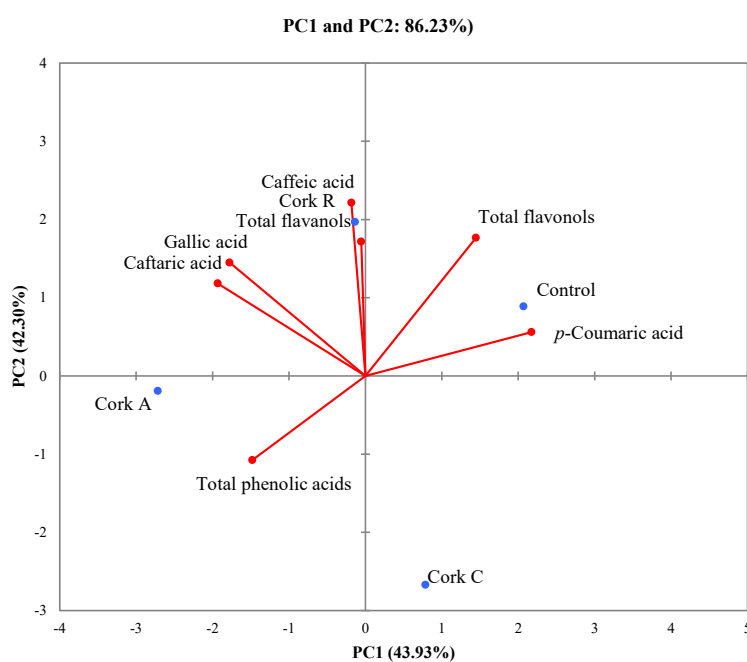


FIGURE 1

PCA biplot illustrating the association of phenolic acids, total phenolics, flavonols and flavanols of wines with different bottle closures.

Oxygen is used by yeast, but is also a substrate for numerous chemical transformations of wines during maturation (Tarko *et al.*, 2020). Additionally, the concentration of extractable phenolic compounds can be potentially affected by factors such as cork area roughness, origin, type, porosity and production steps (Conde *et al.*, 1998). Moreover, phenolic acids permeating from the cork closures can possibly affect the sensory properties of the final wine (Hornedo-Ortega *et al.*, 2020).

The PCA biplot of the first two principal components illustrating the association of phenolic acids and total phenolics of wines with the different cork closures and crown cap closure (control) explained 86.23% of the variation in the data (Fig. 1). The main cause of variation is total flavanols, caffeic, gallic and caftaric acids (as determined by highest squared cosine values, data not shown), which separates Cork R wines from Cork A, Cork C and control wines. Cork A wines were positively associated with total phenolic acids. Control wines (crown closure) were positively associated with *p*-coumaric acid and flavonols. Cork C wines were not associated with any of the measured variables or showed a negative correlation with total flavanols, caffeic, gallic and caftaric acids.

CONCLUSION

The data show that the use of cork during the second fermentation can change the phenolic profile of the wine. Therefore, the cork origin (cork supplier) can bring about subtle differences. Gallic acid was significantly highest in Cork A wines, which indicates the highest contribution of Cork A to the concentration of this compound in wine. The PCA shows that Cork R wines were strongly associated with caffeic, gallic and caftaric acids as well as total flavanols.

For future investigations, it is recommended that phenolic content and physicochemical parameters be measured in the base wine prior to the second fermentation to obtain an understanding of the phenolic compound evolution and physicochemical parameters in the wines during bottle fermentation. In addition, the kinetics of the phenolics released from cork types should be investigated, since the surface roughness of cork can increase the exterior area of the cork closures, therefore, different cork types are assumed to release different concentrations of phenolic compounds and the oxidative state of the phenolic compounds migrating from cork to wine should also be investigated.

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